

incorporate the mutations present in Cry1C.563. Such a mutated crystal protein would, therefore, have modified *both* the α 3/4 loop region and the α 4/5 loop region. For sake of clarity, an " α 3/4 loop region" is intended to mean the loop region between the 3rd and 4th α helices, while an " α 4/5 loop region" is intended to mean the loop region between the 4th and 5th α helices, *etc.* Other helices and their corresponding loop regions have been similarly identified throughout this specification. FIG. 1 illustrates graphically the placement of loop regions between helices for Cry1C.

Preferred mutated *cry1C* genes of the invention are those genes that contain certain key changes. Examples are genes that comprise amino acid substitutions from Arg to Ala or Asp (particularly at amino acid residues 86, 148, 180, 252, and 253); or Lys to Ala or Asp (particularly at amino acid residue 219).

Genes mutated in the manner of the invention may also be operatively linked to other protein-encoding nucleic acid sequences. This will generally result in the production of a fusion protein following expression of such a nucleic acid construct. Both N-terminal and C-terminal fusion proteins are contemplated.

Virtually any protein- or peptide-encoding DNA sequence, or combinations thereof, may be fused to a mutated *cry1C** sequence in order to encode a fusion protein. This includes DNA sequences that encode targeting peptides, proteins for recombinant expression, proteins to which one or more targeting peptides is attached, protein subunits, domains from one or more crystal proteins, and the like.

In one aspect, the invention discloses and claims host cells comprising one or more of the modified crystal proteins disclosed herein, and in particular, cells of the novel *B. thuringiensis* strains EG12111, EG 12121, EG11811, EG11815, EG11740, EG11746, EG11822, EG11831, EG11832, and EG11747 which comprise recombinant DNA segments encoding synthetically-modified Cry1C* crystal proteins which demonstrates improved insecticidal activity against members of the Order Lepidoptera.

Likewise, the invention also discloses and claims cell cultures of *B. thuringiensis* EG12111, EG12121, EG11811, EG11815, EG11740, EG11746, EG11822, EG11831, EG11832, and EG11747. Such cell cultures may be biologically-pure cultures consisting of a single strain, or alternatively may be cell co-cultures consisting of one or more

strains. Such cell cultures may be cultivated under conditions in which one or more additional *B. thuringiensis* or other bacterial strains are simultaneously co-cultured with one or more of the disclosed cultures, or alternatively, one or more of the cell cultures of the present invention may be combined with one or more additional *B. thuringiensis* or other bacterial strains following the independent culture of each. Such procedures may be useful when suspensions of cells containing two or more different crystal proteins are desired.

The subject cultures have been deposited under conditions that assure that access to the cultures will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122. The deposits are available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposits will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, *i.e.*, they will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the finishing of a sample of the deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the cultures. The depositor acknowledges the duty to replace the deposits should the depository be unable to furnish a sample when requested, due to the condition of the deposits. All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

Cultures of the strains listed in Table 2 were deposited in the permanent collection of the Agricultural Research Service Culture Collection, Northern Regional Research Laboratory (NRRL) under the terms of the Budapest Treaty:

TABLE 2

STRAINS DEPOSITED UNDER THE TERMS OF THE BUDAPEST TREATY

| Strain | Protein/Plasmid | Accession Number | Deposit Date |
|---------------------------------|-------------------|---------------------|---------------|
| <i>B. thuringiensis</i> EG11740 | Cry1C.563 | NRRL B-21590 | Jun. 25, 1996 |
| <i>B. thuringiensis</i> EG11746 | Cry1C.579 | NRRL B-21591 | Jun. 25, 1996 |
| <i>B. thuringiensis</i> EG11811 | Cry1C-R148A | NRRL B-21592 | Jun. 25, 1996 |
| <i>B. thuringiensis</i> EG11747 | Cry1C.499 | NRRL B-21609 | Aug. 2, 1996 |
| <i>B. thuringiensis</i> EG11815 | Cry1C-R180A | NRRL B-21610 | Aug. 2, 1996 |
| <i>B. thuringiensis</i> EG11822 | Cry1C-R148A | NRRL B-21638 | Oct. 28, 1996 |
| <i>B. thuringiensis</i> EG11831 | Cry1C-R148A | NRRL B-21639 | Oct. 28, 1996 |
| <i>B. thuringiensis</i> EG11832 | Cry1C-R148D | NRRL B-21640 | Oct. 28, 1996 |
| <i>B. thuringiensis</i> EG12111 | Cry1C-R148A-K219A | NRRL B-XXXXXX | Nov. XX, 1997 |
| <i>B. thuringiensis</i> EG12121 | Cry1C-R148D-K219A | NRRL B-XXXXXX | Nov. XX, 1997 |
| <i>E. coli</i> EG1597 | pEG597 | NRRL B-18630 | Mar. 27, 1990 |
| <i>E. coli</i> EG7529 | pEG853 | NRRL B-18631 | Mar. 27, 1990 |
| <i>E. coli</i> EG7534 | pEG854 | NRRL B-18632 | Mar. 27, 1990 |

2.2 METHODS FOR PRODUCING CRY1C* PROTEIN COMPOSITIONS

The modified Cry1* crystal proteins of the present invention are preparable by a process which generally involves the steps of: (a) identifying a Cry1 crystal protein having one or more loop regions between two adjacent α helices or between an α helix and a β strand; (b) introducing one or more mutations into at least one of these loop regions; and (c) obtaining the modified Cry1* crystal protein so produced. As described above, these loop regions occur between α helices 1 and 2, α helices 2 and 3, α helices 3 and 4, α helices 4 and 5, α helices 5 and 6, and α helices 6 and 7 of domain 1 of the crystal protein, and between α helix 7 of domain 1 and the β strand 1 of domain 2.

Preferred crystal proteins which are preparable by this claimed process include the crystal proteins which have the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59, or SEQ